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P. falciparum Merozoite Surface Protein-1 (MSP-1) is a leading erythrocytic-stage vaccine candidate. It is proteolytically processed to several fragments, and may play a role in binding and/or invasion of erythrocytes. During erythrocyte invasion secondary processing on MSP-1_{42} gives a 33kDa and a 19kDa fragment (MSP-1_{19}). Although, MSP-1_{19} is a target for parasite inhibitory mAbs and protective immunity, it appears to lack T-helper epitopes. Since antibody is likely the effector mechanism induced by MSP-1_{19}, it is important to insure that recombinant vaccines based upon this antigen be folded correctly and contain T-helper epitopes that will enhance induction of humoral responses. We developed recombinant MSP-1_{42} that was structurally correct based on positive reactivities with conformation-dependent mAbs, including 12.10, 12.8. 7.5, 7.2, and 1E1. Purification over three chromatographic steps that include nickel affinity chromatography, Q-substituted and CM-substituted resins, yielded protein that was 95% pure and exceeded FDA endotoxin standards. Mice seroconverted following immunization with recombinant MSP-1_{42}. Safety and immunogenicity testing in Rhesus monkeys revealed that MSP-1_{42} /SBAS2, was safe and induced MSP-1_{42}-specific antibody responses.

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FOREWORD

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Evelyn Angus 5/4/00

PI - Signature Date

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INTRODUCTION

The objective of this work was to produce GMP-grade, properly folded *E. coli* expressed MSP-1_{42} antigen for a human Phase I clinical trial. *In vitro* studies have shown that protective epitopes on MSP-1 are present on the C-terminal MSP-1 cleavage product, MSP-1_{42} (Egan, 1999). However, C-terminal MSP-1_{42} contains complex tertiary structure. Thus, correct presentation of relevant epitopes on MSP-1_{42} is dependent on proper folding and conformation. Plasmid containing the MSP-1_{42} gene sequence was used to transform *E. coli* and express recombinant MSP-1_{42}. Purification process development of recombinant MSP-1_{42} included a metal chelating resin, an anion exchanger, followed by cation exchange. Purified MSP-1_{42} was characterized for correct structure using conformation-dependent mAbs specific for epitopes on either MSP-1_{19} or MSP-1_{33}. MSP-1_{42} reconstituted with adjuvant was used to immunize mice and Rhesus monkeys to study the induction of MSP-1 specific antibodies.

Background Review

The mosquito-born parasite *Plasmodium falciparum* is the leading cause of clinical malaria. Each year greater than 300 million cases of clinical malaria are reported. Alternative approaches to mosquito vector-control strategies and chemotherapeutics, include development of recombinant antigens for use as immunogens to induce specific immunity against native malarial antigens. Several vaccine candidates have been identified from each of the parasite's developmental stages. Vaccines that are derived from malaria erythrocytic-stage antigens are of special interest because erythrocytic stages are the only

confirmed targets of natural immunity among individuals from malaria endemic regions. The development of protective antibodies against malaria antigens is supported by studies in which immunoglobulin from individuals protected from *P. falciparum* infection was passively transferred into Thai patients infected with malaria (Sabchareon, 1991). Parasitemia in these individuals treated with IgG was reduced.

The major merozoite surface protein-1, MSP-1, is among the leading erythrocytic stage vaccine candidates [Diggs, 1993]. The objective of erythrocytic stage vaccines is to diminish the level of parasitemia in the bloodstream and thus reduce the severity of disease. MSP-1 has been studied extensively [Holder, 1988, Miller, 1993], however, its specific role in erythrocyte binding and invasion still remains uncertain [Holder and Blackman, 1994]. The 195kDa MSP-1 is processed to polypeptides of 83, 28-30, 38-45 and 42 kDa during merozoite development and merozoite-specific antibodies recognize these proteolytic forms [Holder and Freeman, 1984; Lyon, 1986; Holder, 1987]. These fragments remain associated to the merozoite surface through non-covalent interactions [McBride and Heidrich, 1987; Lyon, 1987] and are attached to the merozoite surface through the C-terminal 42 kDa fragment ($MSP\text{-}1_{42}$). A secondary-processing event required for invasion yields a 33-kDa fragment and a 19 kDa C-terminal fragment ($MSP\text{-}1_{19}$) [Blackman, 1991]. Immediately following invasion, $MSP\text{-}1_{19}$ is present on ring-forms in the newly invaded erythrocyte [Blackman, 1990].

Evidence for the use of MSP-1, and especially the C-terminal fragments, $MSP\text{-}1_{42}$ and $MSP\text{-}1_{19}$, as components of erythrocytic stage malaria vaccines is

extensive. MSP-1_{19}-specific mAbs inhibit *P. falciparum* growth *in vitro,* [Blackman, 1990], and passively protect mice against infection with *P. yoelii,* [Majarian, 1984; Ling, 1994]. Immunization of monkeys with native MSP-1, [Siddiqui, 1987], baculovirus-expressed recombinant MSP-1_{42} [Chang, 1996], or *S. cerevisiae*-secreted MSP-1_{19} (EVE-MSP-1_{19}) from *P. falciparum* [Kumar, 1995], protect against a homologous challenge. Similarly, *E. coli*-expressed MSP-1_{19} from *P. yoelii,* [Holder, 1994; Burns, 1989] protects against a homologous murine challenge. Anti-sera raised against recombinant MSP-1_{42} [Chang, 1992], or MSP-1_{19} [Lyon and Haynes, unpublished] inhibit *P. falciparum* growth *in vitro.* The MSP-1_{19}-specific mAbs that either protect against infection *in vivo* [Burns, 1989], or inhibit parasite growth *in vitro* [Blackman, 1990], are specific for discontinuous epitopes since they do not react with disulfide-reduced MSP-1_{19} [McBride and Heidrich, 1987; Farley and Long, 1995]. MSP-1_{19} folds into a complex disulfide bridged structure (Holder, et al. ref) with homology to epidermal growth factor (EGF) at the level of the cysteines [Blackman, 1991]. Within a span of 100 amino acid, two EGF-like domains contain six cysteine residues that form three disulfide bridges per domain.

Body

The objective was to produce a recombinant MSP-1_{42} expressed from *E. coli* that could be tested for safety and immunogenicity in a Phase I clinical trial. To meet this objective, a plasmid that expressed properly folded soluble MSP-1_{42} was constructed using recombinant DNA technology. The gene fragment containing MSP-1_{42} was cloned into a modified *E. coli* expression vector, pET32a

(Novagen). The vector was modified so that only 17 nonMSP-1_{42} amino acids were fused to the N-terminus of MSP-1_{42}. These residues included a six-histidine tag for metal chelating chromatography, and 11 linking amino acids. The vector was modified to contain the gene for tetracycline resistance for selection during fermentation. The cloned product was tested for soluble expression of MSP-1_{42} in *E. coli* BL21 (DE3). Induction of protein expression proceeded at 25°C to minimize the rates of protein translation and thus reduce the levels of insoluble protein expressed.

MSP-1_{42} was purified to greater than 95% over three chromatographic steps under GMP standards in June 1999 at the Walter Reed Army Institute of Research, Department of Biologics Research, GMP Pilot Production Plant, Forest Glen, MD. The purification process was developed using *E. coli* paste derived from 300L GMP fermentation's produced in June 1998 and June 1999 at the Walter Reed Army Institute of Research, Department of Biologics Research, Pilot Plant, Forest Glen, MD. Cleared lysates were applied to a Ni^{+2}-NTA Superflow resin for affinity purification of His-tagged MSP-1_{42}. The lysate was applied in a pH 6.2 buffer and washed extensively to reduce the level of free endotoxin. Bound MSP-1_{42} eluted with imidazole. Eluted MSP-1_{42} protein from the Ni^{+2}-NTA resin was greater than 50% pure relative to *E. coli* proteins. The eluted protein was applied to a SuperQ anion exchanger and the flow-through containing MSP-1_{42} was collected. The final chromatographic step was on a cation exchanger, CM 650M. MSP-1_{42} bound the CM column and was eluted with NaCl. The protein was concentrated and the buffer was exchanged using

an ultrafiltration unit. The final product was diluted into buffer containing excipients and lyophilized into single dose vials. The yield from this production was approximately 10,000 doses of purified MSP-1_{42} antigen with 600 single dose vaccine vials produced to support safety and immunogenicity studies in a Phase I clinical trial.

The *E. coli* expressed MSP-1_{42} was analyzed for total protein and purity by SDS-PAGE Coomassie Blue staining. MSP-1_{42} was purified to greater than 95% purity as shown by Coomassie Blue staining (Figure 1A) and scanning densitometry (data not shown). Although the MSP-1_{42} protein was highly purified, several protein fragments were visible under nonreduced conditions. The major Coomassie Blue stainable band was MSP-1_{42}. Also observed were some minor truncated forms (36kD and 38kD) that migrated below 42kDa and higher molecular weight aggregates that migrated at 90kDa and greater. All Coomassie Blue stainable protein bands were reactive with the MSP-1_{33} specific mAb 7F1 (Figure 1A). In contrast, the 36kD and 38kD fragments did not react with the MSP-1_{19} specific mAb 12.10 or any other MSP-1_{19} specific mAb tested (data not shown). These results indicated that the 36kD and 38kD fragments lacked part of MSP-1_{19}. Full-length MSP-1_{42} and higher molecular weight aggregates were reactive against all the MSP-1 specific mAbs shown in Figure 1B, including the inhibitory mAbs that inhibit processing and invasion (12.10, 12.8) and the blocking mAbs, that block inhibitory mAbs activity (2.2, 7.5, 1E1).

To measure the induction of MSP-1_{42} specific antibodies, Balb/C mice were immunized with MSP-1_{42} plus SBAS2 adjuvant (SmithKline Beecham

Biologics, Belgium). A dose titration of antigen was performed to identify the minimum dose necessary for positive seroconversion following a single immunization. Mice were immunized at 3-week intervals subcutaneously with 0.1, 0.3 or 1.0 μg's of MSP-1_{42}/SBAS2. Pre-immune sera were collected, and immune sera were collected 2 weeks following each immunization. Sera were analyzed by ELISA against yeast expressed MSP-1_{19} coated plates. Seroconversion rates following the first and second immunization are shown at the 99% confidence interval (Figure 2). At the highest dose, which was 1.0 μg, we observed 100% seroconversion following one immunization, while at the lowest dose, which was 0.1 μg, we observed seroconversion only following two immunizations. At the 0.3 μg and 1.0 μg doses, 20% and 100% of mice in each group seroconverted following one immunization, respectively. While at the lowest dose only 60% of the mice seroconverted following the second immunization.

These mice were boosted at 3-week intervals for a total of 5 immunizations and sera from these mice were collected and analyzed for induction of MSP-1_{19} specific antibodies following each immunization. Mice immunized with the highest dose, which was 1.0 μg, produced high antibody titers following the 2nd immunization and this titer was not boosted appreciably following further immunization. Mice immunized with the lowest dose, which was 0.1 μg, eventually produced the same antibody titer as mice immunized with the higher doses (Figure 3). We observed good seroconversion rates in mice after one or two immunizations. After five immunizations the mean antibody titers in these mice exceeded 1:80,000 independent of dose.

Immune sera from mice that were immunized 5 times with 1.0 μg MSP-1_{42}/SBAS2 were tested by IFA using serum dilutions of 1:1,000 and 1:10,000. At the 1:1,000 dilution, all of the sera were IFA positive. At the 1:10,000 dilution, 70% of the sera were IFA positive (Table 1). These data indicated that mice immunized with MSP-1_{42}/SBAS2 induced antibodies that were able to react with parasite produced antigen.

Rhesus monkeys were immunized with 50 μg doses, the anticipated human dose, of MSP-1_{42}/SBAS2 or with MSP-1_{42}/alum to access safety and immunogenicity. Each group comprised of eight Rhesus monkeys, which were immunized intramuscularly at 0, 1 month, and 3-month intervals. For both groups no adverse local responses were observed and all biochemical and hematological laboratory tests were normal. Sera were collected 2 weeks following each immunization and antibody titers were analyzed by ELISA with plates coated with yeast expressed MSP-1_{19}. Antibody titers were reported in ELISA units. For the group in which antigen was formulated with SBAS2, MSP-1 specific antibodies were induced after the 2nd immunization, and peaked at 10,000 ELISA units 2 weeks following this immunization. In this group, the antibody titers declined steadily over the next 7 weeks. Two weeks following the 3rd immunization, antibody titers peaked at a mean of 19,000 ELISA units. Antibody titers induced by immunization with MSP-1_{42}/SBAS2 were at least 10 fold greater than those induced by immunization with MSP-1_{42}/Alum (Figure 4).

Discussion

The production of an inexpensive, safe and efficacious vaccine for malaria has proven to be a difficult task. Expression of malaria antigens in heterologous expression systems has been difficult due to the substantial codon bias toward A-T rich sequences. Typically these antigens have either been expressed in insoluble forms or have been expressed poorly within the heterologous expression hosts. Here we have described the development of a fermentation and purification process for the production of an MSP-1_{42} subunit vaccine that was safe and induced MSP-1 specific antibodies in mice and Rhesus monkeys. These data support the use of MSP-1_{42}/SBAS2 in a Phase I human trial.

The purification process developed for MSP-1_{42} yielded a highly purified product that has met all FDA standards for a subunit vaccine product to be tested in a Phase I safety trial. Endotoxin levels in the purified protein were significantly below the FDA acceptable levels (FDA; 350 EU/dose/70kg human, Our Process; 9.14 EU/dose/70kg human). Residual levels of all chemicals used in the purification process were quantified and determined to be within acceptable levels set as production specifications.

Even though MSP-1_{42} was highly purified, some proteolysis occurred within the C-terminal MSP-1_{19} fragment, most likely during expression in the *E. coli* host. Long term stability studies on purified antigen stored at –80°C, however, have shown no additional antigen instability (data not shown). All Coomassie Blue detectable bands, full length MSP-1_{42}, C-terminal truncated forms, and high molecular weight aggregates were reactive with MSP-1 specific

mAbs. Previous studies have shown that the induction of antibody responses to epitopes on MSP-1_{19} correlated with clinical immunity to malaria. Therefore, properly formed disulfide-dependent conformational epitopes on recombinant MSP-1_{19} or MSP-1_{42} molecules are required for the induction of protective antibody responses. The *E. coli* expressed recombinant MSP-1_{42} appeared to contain some structurally correct epitopes because it reacted with 8 MSP-1_{19} specific mAbs including functional mAbs classified as growth or invasion inhibitory (mAb 12.10, 12.8) and blocking inhibitory mAbs (mAb 7.5, 2.2, 1E1). Immune sera from mice immunized with the highest dose of MSP-1_{42}/SBAS2 were reactive on methanol fixed parasites by IFA's suggesting that immunization with recombinant MSP-1_{42} induced antibodies to some native MSP-1 structures. These data support the use of this bacterial expressed MSP-1_{42} antigen in a clinical trial because it may contain some relevant epitopes for the induction of protective antibodies.

Currently, the only malaria vaccine tested in a Phase I trial that has reproducibly protected volunteers at the 50-70% level has been the RTS,S/SBAS2 vaccine developed by Smithkline Rixensart, Belgium (Stoute, 1997). Substantial safety and efficacy data support the combination of the SBAS2 adjuvant with malaria antigens for the induction of protective immune responses. Our studies in Rhesus monkeys immunized with MSP-1_{42}/SBAS2 showed no adverse biochemical or local reactions to this antigen/adjuvant combination. In addition, MSP-1_{42}/SBAS2 was highly immunogenic in the Rhesus monkeys as well as Balb/C mice. After three immunizations the mean antibody

titers in Rhesus monkeys exceeded 1:19,000. MSP-1_{42}/SBAS2 induced at least ten times more MSP-1 specific antibodies than MSP-1 /Alum. MSP-1 specific antibodies induced by this vaccine were predominantly against MSP-1_{42} (the immunizing antigen) compared to MSP-1_{33} and MSP-1_{19} as would be expected. The SBAS2 formulated vaccine yielded consistently the highest levels of antibodies over the Alum formulation in this immunization regimen. Additional data from the Rhesus monkeys following their fourth immunization and fifth immunizations (ongoing studies) will provide information on the ability of the MSP-1_{42}/SBAS2 vaccine to further boost MSP-1 specific antibody.

Key Research Accomplishments

1. Produced 300L of GMP fermentation paste from *E.coli* Expressed MSP-1_{42}. (June 1999)
2. Produced approximately 10,000 doses of MSP-1_{42} bulk antigen for future clinical trials. (June 1999)
3. Produced 600 single dose vaccine vials containing MSP-1_{42} antigen for formulation with adjuvant. (June 1999)
4. Complete characterization of manufacturing process of the GMP purified MSP-1_{42} antigen that meets FDA standards for a product to be used in humans.
5. Documentation of the production and characterization of MSP-1_{42} as written in an IND application for the FDA.
6. Initiated the purification process development for an alternative allelic form of the MSP-1_{42} (3D7) strain, the MSP-1_{42} (FVO) strain to be used in studies investigating induction of strain specific immunity.
7. Completed two 300 L GMP Fermentation processes on MSP-1_{42} (FVO). (February 2000, March 2000)
8. Prepared reagents, plasmid constructs, protein products, necessary to further characterization the functional activities of MSP-1_{42}.

Reportable Outcomes

1. Manuscript in preparation:

Evelina Angov, Barbara Aufiero, Michel Van Handenhove, Christian Ockenhouse, Kent Kester, Doug Walsh, Jana McBride, Gray Heppner, Ripley W. Ballou, Carter Diggs, Jeffrey Lyon, "Process Development and Immunogenicity Studies on a Clinical Grade *E. coli* Expressed *P. falciparum* Merozoite Surface Protein-1_{42} (3D7) Vaccine"

2. Guest Seminar speaker, October 1999:
   ATCC, Manassa, Virginia
   Topic: Purification and Characterization of rMSP1_{42}, an Erythrocytic Stage Malaria Vaccine Candidate

3. ASTMH Conference, Washington DC November 1999
   Oral Presentation:
   Topic: Process Development for Clinical Grade *Plasmodium falciparum* MSP1_{42} (3D7) Expressed In *E. coli*

4. Poster Presentation, Lorne, Australia
   Meeting: Molecular Approaches to Malaria
   Abstract Title: Characterization of clinical grade *P. falciparum* MSP1_{42} (3D7) expressed in *E. coli*

Conclusions

These data support the use of this *E.coli* expressed MSP-1_{42} antigen when adjuvanted with SBAS2 in a Phase I human trial. The data show that the recombinant MSP-1_{42} appears to be structurally correct because it was reactive with 8 MSP-1_{19} specific monoclonal antibodies. Reactivity with disulfide dependent epitopes on MSP-1_{19} can predict some correct structure on the recombinant MSP-1_{42}.

Safety and immunogenicity studies in Balb/c mice and rhesus monkeys support the use of recombinant MSP-1_{42} adjuvanted with SBAS2. Both animal models were able to seroconvert to MSP-1 positive specific antibodies. The antibody titers that were induced reflected the induction of good MSP-1 specific antibody responses. After five immunizations the mean antibody titers in mice exceeded 1:80,000 independent of dose. While after three immunizations the mean antibody titers in Rhesus monkeys exceeded 1:19,000.

These data support the further evaluation of MSP-1_{42} recombinant antigen as a promising erythrocytic-stage malaria vaccine candidate. Future studies include characterization of the specific types of antibodies induced (blocking versus processing inhibitory) by vaccination with this antigen. These assays will be developed using the immune sera from the Balb/C mice and Rhesus monkeys. Pending FDA approval, a CY 2000 3rd quarter start date for a Phase I safety and immunogenicity study at the WRAIR is planned. In addition, new production lots of the MSP-1_{42} antigen will be characterized for antigen stability, for structure relative to monoclonal antibody binding, for the induction of MSP-1

immunogenicity, and tested for safety relative to the original production lot. These data will support the decision process for future productions of MSP-1 antigens as malaria vaccine candidates.

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Figure Legends

Figure 1:
A). SDS-PAGE analysis of GMP purified MSP-1_{42} for total protein by Coomassie Blue staining and Immunoblotting with MSP-1_{19} specific mAbs under nonreducing conditions. A) Coomassie Blue staining of nonreduced protein at 10 μg. Immunodetection with mAbs 7F1 and 12.10 on 10 μg of Purified MSP-1_{42}. B) mAb reactivities, Shown is a list of monoclonal antibodies used to measure MSP-1_{19} specific and MSP-1_{33} specific reactivities on GMP produced MSP-1_{42}.

Figure 2:
ELISA assays to measure Seroconversion in mice.
Antibody titers were reported as the dilution of serum that produced 1 absorbance unit in the ELISA (ELISA units). Balb/C mice immunized with 0.1, 0.3, and 1.0ug's of MSP-1_{42}/SBAS2. Antibody titers are reported at a 99% confidence interval.

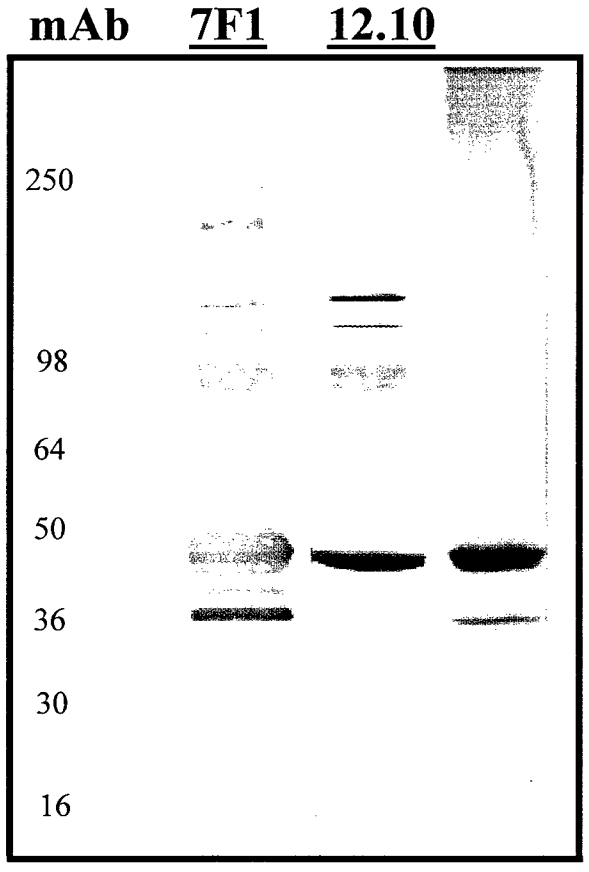
Figure 3:
ELISA assays to measure Immunogenicity in mice.
Balb/C mice were immunized with 0.1, 0.3, and 1.0ug's of MSP-1_{42}/SBAS2. MSP-1 specific antibodies are detected against plates coated with yeast MSP-1_{19}. Antibody titers are reported at a 99% confidence interval. Antibody titers were reported as the dilution of serum that produced 1 absorbance unit in the ELISA (ELISA units).

Figure 4:
Immunogenicity in Rhesus. ELISA assay on MSP-1_{42} formulated with adjuvants, SBAS2 and Alum. Rhesus monkeys were immunized with 50 ug's of MSP-1_{42}/adjuvant. MSP-1 specific antibodies were detected against plates coated with yeast MSP-1_{19}.

Table 1:
Immunogenicity in mice measured by IFA
Sera from mice immunized with 1.0 μg doses of MSP-1_{42}/SBAS2 were tested by IFA against methanol fixed parasites. Values are shown as dilutions of mouse sera and their ability to react on parasite lysates.

Figure 1

A.



B.

mAbs	Specificity	Reactivity
12.10	MSP-1_{19} Domain 1 + 2	++
12.8	MSP-1_{19} Domain 1	++
7.5	MSP-1_{19} Domain 1	++
2.2	MSP-1_{19} Domain 1	++
5.2	MSP-1_{19}	++
1E1	MSP-1_{19}	++
7F1	MSP-1_{33}	++
3H7	MSP-1_{19}	++
3B10	MSP-1_{19}	++

Figure 2

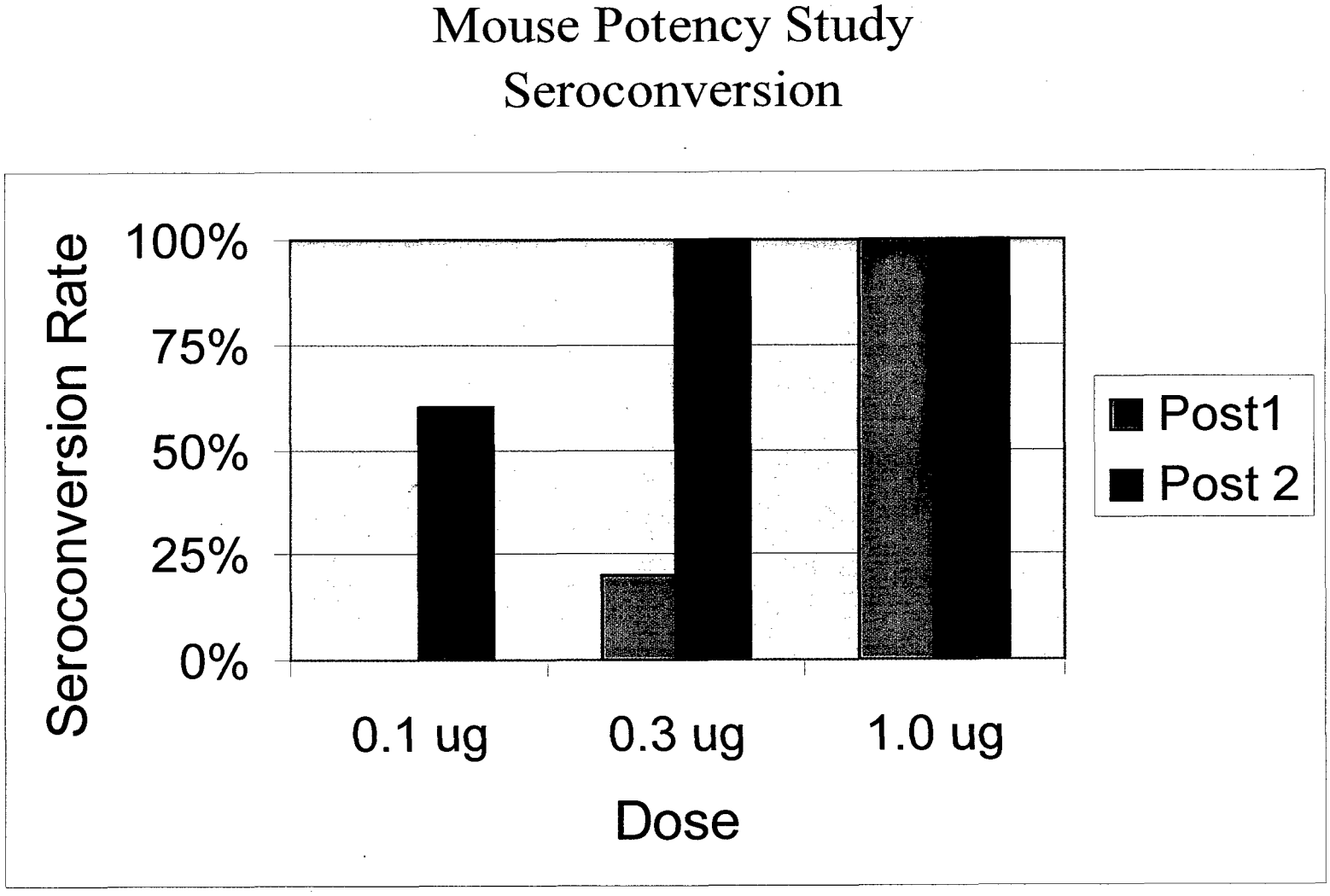

Mouse Potency Study
Seroconversion
Seroconversion Rate
100%
75%
50%
25%
0%
0.1 ug
0.3 ug
1.0 ug
Dose
Post1
Post 2

Table 1

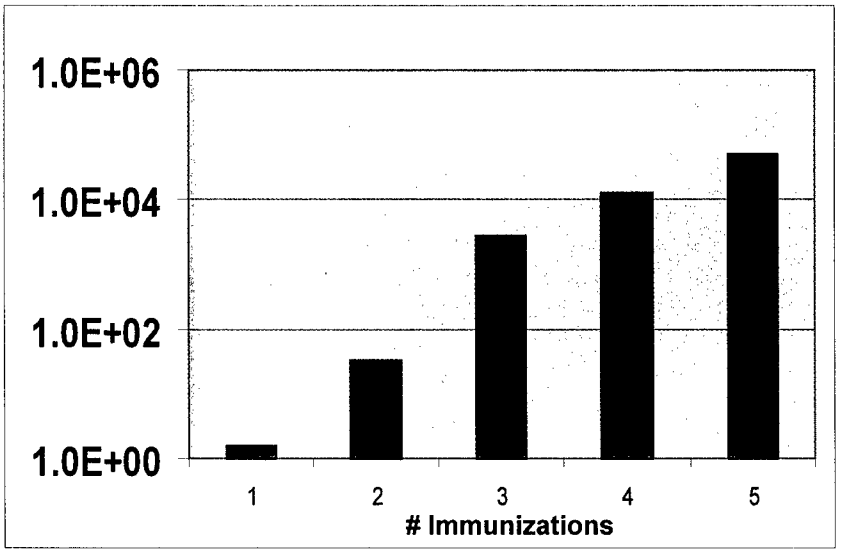
Mouse Immunogenicity
by IFA

	Pre-immune	Post 5		
Dilution	1:250	1:1,000	1:3,000	1:10,000
# Seroconverted	0/10	10/10	9/10	7/10

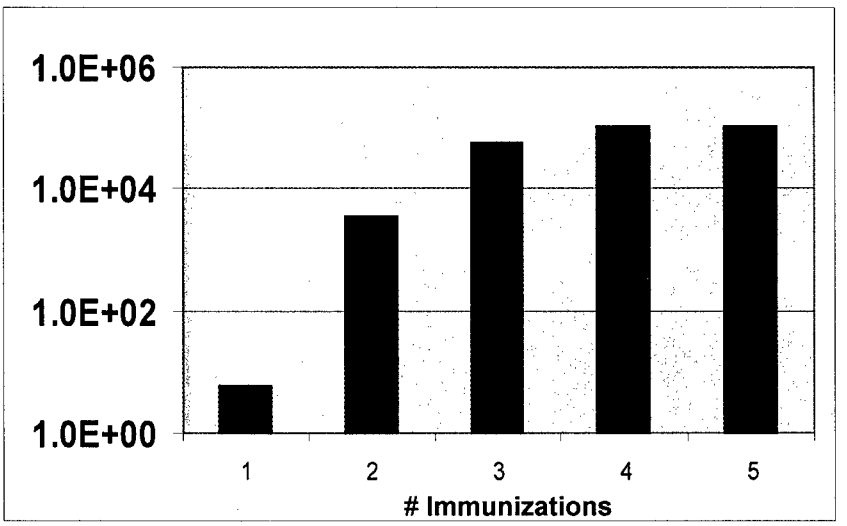
Figure 3

Mouse Immunogenicity (ELISA)

0.1ug Dose



0.3ug Dose



1.0ug Dose

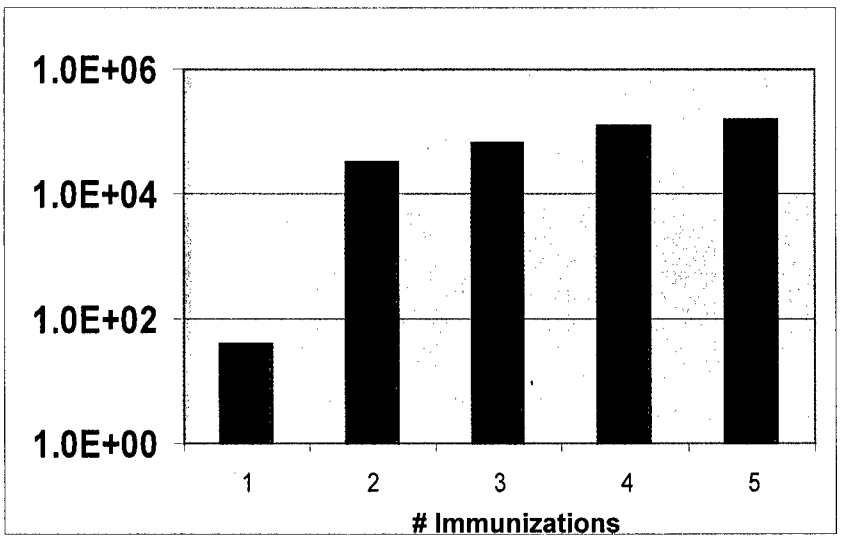
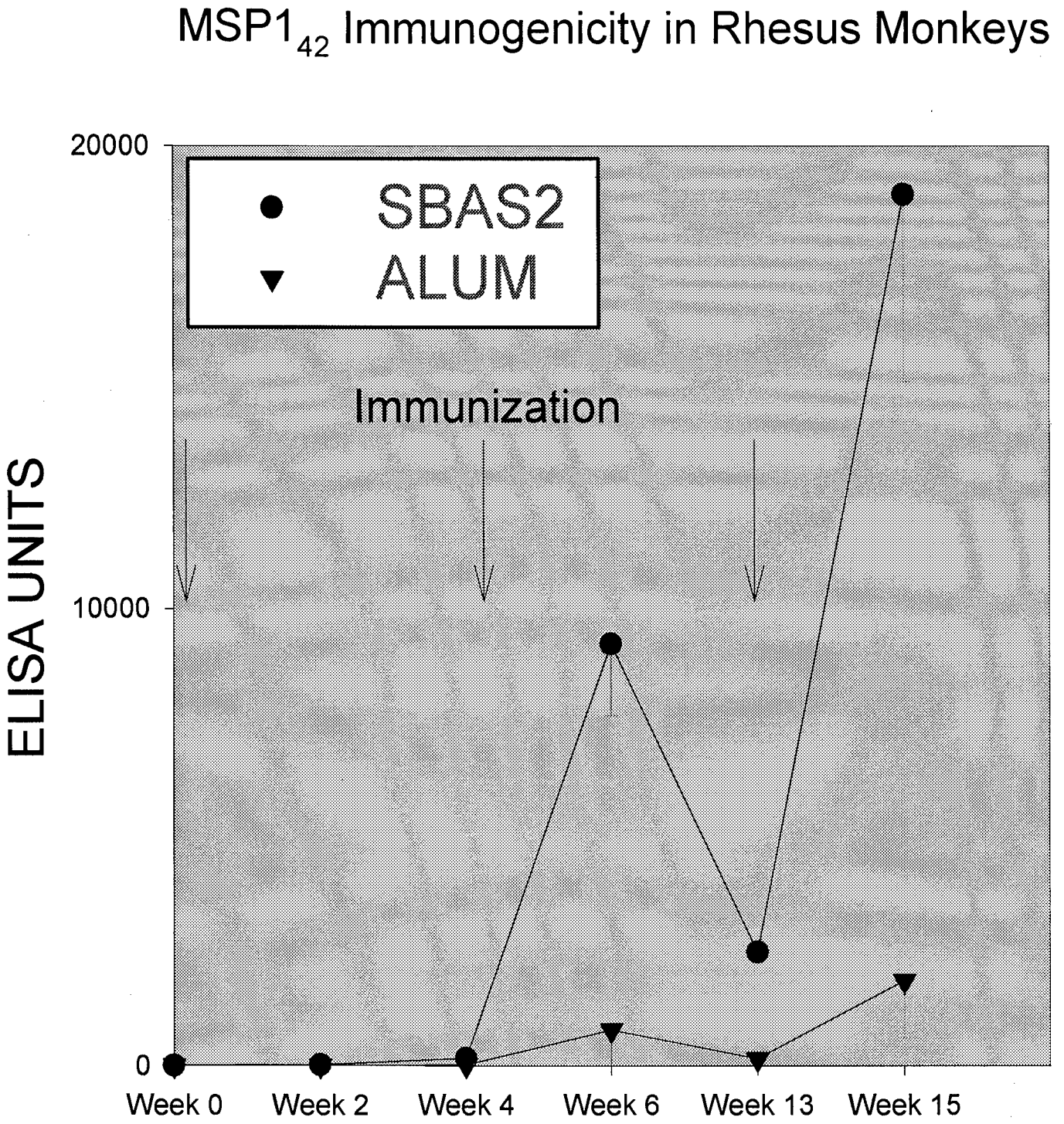


Figure 4


MSP1 42 Immunogenicity in Rhesus Monkeys
20000
10000
0
ELISA UNITS
SBAS2
ALUM
Immunization
Week 0
Week 2
Week 4
Week 6
Week 13
Week 15

Process development for clinical grade *P. falciparum* $MSP1_{42}$ (3D7) expressed in *E. coli*.

E Angov, B Aufiero, C Ockenhouse, K Kester, D Walsh, S Pichyangkul, M Van Handenhove[2], WR Ballou, C Diggs[1], J Lyon

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The merozoite surface protein-1 (MSP1) of *P. falciparum* is a leading erythrocytic-stage vaccine candidate. It is a 195kDa protein that is proteolytically processed to several fragments, and may play a role in binding and/or invasion of erythrocytes by merozoites. In conjunction with erythrocyte invasion, the most distal C-terminal fragment ($MSP1_{42}$) undergoes secondary processing giving a 33kDa and a 19kDa fragment ($MSP1_{19}$). Although the latter is a target for parasite inhibitory mAbs and protective immune responses, it appears to lack T-helper epitopes. Since antibody is most likely the effector mechanism induced by $MSP1_{19}$, it is important to insure that recombinant vaccines based upon this antigen be folded correctly and contain T-helper epitopes that will enhance induction of humoral responses. To fulfill these objectives we developed recombinant $MSP1_{42}$ molecules that were structurally correct and developed fermentation and purification processes that could advance this product into human clinical trials. Bacterial expression of $MSP1_{42}$ from the pET T7 driven promoter-expression system resulted in soluble $MSP1_{42}$ that is immunoreactive with several functional $MSP1_{19}$-specific mAbs, including mAbs 12.10, 12.8. 7.5, 7.2, and 1E1. Purification over three chromatographic steps that include Ni^{+2} affinity chromatography, anion exchange on a Q-substituted resin and cation exchange on a CM-substituted resin, yields protein that is 95% pure and exceeds FDA endotoxin standards. The final lyophilized product is stable. Mice seroconverted following immunization with recombinant $MSP1_{42}$ adjuvanted with SBAS2. Safety and immunogenicity testing in Rhesus monkeys reveals that $MSP1_{42}$ in combination with SBAS2 is safe and induces $MSP1_{42}$ specific antibody responses.

PROCESS DEVELOPMENT FOR CLINICAL GRADE /PLASMODIUM FALCIPARUM/ MSP1/42 (3D7) EXPRESSED IN /E. COLI/.

The merozoite surface protein-1 (MSP1) of /P. falciparum/ is a leading erythrocytic-stage vaccine candidate. It is a 195kDa protein that is proteolytically processed to several fragments, and may play a role in binding and/or invasion of erythrocytes by merozoites. In conjunction with erythrocyte invasion, the most C-terminal fragment (MSP1/42) undergoes secondary processing giving a 33kDa and a 19kDa fragment (MSP1/19\). Although the latter is a target for parasite inhibitory monoclonal antibodies and protective immune responses, it appears to lack T-helper epitopes. Since antibody is most likely the effector mechanism induced by MSP1/19\, it is important to insure that recombinant vaccines based upon this antigen be folded correctly and contain T-helper epitopes that will enhance induction of humoral responses. To fulfill these objectives we developed recombinant MSP1/42\ molecules that were structurally correct and developed fermentation and purification processes that could advance this product into human clinical trials. Bacterial expression of MSP1/42\ from the pET T7 driven promoter-expression system results in soluble MSP1/42\ that is immunoreactive with several functional MSP1/19\-specific mAbs, including mAbs 12.10, 12.8. 7.5, 7.2, and 1E1. Purification over three chromatographic steps that include nickel affinity chromatography, anion exchange on a Q-substituted resin and cation exchange on a CM-substituted resin, yields protein that is 95% pure and exceeds FDA endotoxin standards. The final lyophilized product is stable. Mice seroconverted following immunization with recombinant MSP1/42\. Safety and immunogenicity testing has been initiated in Rhesus monkeys.

BIOGRAPHY BRIEF

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ILIR Relevant Professional Employment and/or Experience:
Sept 1989-Jan 1995: Intramural Research Training Award Fellow (IRTA), Genetics and Biochemistry Branch, National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), NIH, DNA Recombination, *Thermus aquaticus recA.*

Mar 1995-Present: Molecular Biologist/Biochemistry, Contract, Department of Immunology, WRAIR, Malaria Vaccine Development Program, USAID; Molecular Biology and Biochemistry.

ILIR Relevant Publications or Inventions:

Yu, X., Angov, E., Camerini-Otero, R.D., and E.H. Egelman. 1995. Structural polymorphism of the RecA protein from the thermophilic bacterium *Thermus aquaticus.* Biophys. J. 69:2728-38.

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